REMARKS

Claims 1-7, 11-17, 59-61, 65-68 and 71-74 presently appear in this case. No claims have been allowed. The official action of October 4, 2007, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to a method of altering gene expression in a population of human embryonic stem cells by introducing into the population of human embryonic stem cells a polynucleotide that contains a gene expression altering sequence. It is possible to obtain a transfection efficiency greater than that obtainable by means of electroporation, preferably under conditions using a single 625 V/cm pulse at room temperature, by use of a cationic non-lipid polymer transfection reagent, preferably a linear polymer of polyethyleneimine.

The telephone interview between Examiner Ton and the undersigned attorney conducted on April 3, 2008, is hereby gratefully acknowledged. In this telephone interview, applicant advised the examiner that it wished to limit the claims to the subclass of transfection reagents that have specifically been found to be effective in the present specification, i.e. the subclass that includes Exgen 500. The undersigned advised the examiner of the reasons why it was believed that this entire subclass of polymers was supported. The arguments will be

repeated hereinbelow. The undersigned also asked the examiner if she would accept claims limited to the transfection reagent being a linear polymer of ethyleneimine as the specification discloses that this is the chemical designation of Exgen transfection reagents. The examiner stated that she would not be able to make a decision on the telephone, but that she would keep the arguments in mind at the time reviews our written response.

Claims 67, 69 and 70 have been objected to because the claims recite "A method of claim 65" while there is only one method in the independent claim. The examiner suggests that applicants amend the claims to recite "The method of".

This objection is totally inappropriate and untenable. The independent claims are not directed to one method. The independent claims are generic and cover a myriad of specific methods all of which fall within the scope of the generic method claim. It is perfectly appropriate to limit the scope of a generic method claim to a sub-generic method by calling the dependent claim "A method [within the genus of methods set forth in claim 1]." Dependant claims beginning with the word "A" are acknowledged and used as acceptable examples in the MPEP at 608.01(n). Literally hundreds of thousands of patents use this language. This type of objection should never be made by an examiner. Nevertheless, in order to obviate this objection,

claims 69 and 71 have been written using "The" rather than "A". Reconsideration and withdrawal of this objection are therefore respectfully urged.

Claims 1-9, 11-17 and 59-70 have been rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement. The examiner states that this is a new matter rejection. The examiner states that the specification provides support for Exgen 500, which produces the claimed result of a transfection efficiency greater than that obtainable by electroporation. The examiner states that because the disclosure does not support that all of the reagents claimed have a transfection efficiency greater than that obtained by electroporation, this would indicate that applicants were not in possession of the claimed invention. The examiner states that one of skill in the art would recognize that the specification only provides a description for Exgen 500 as a transfection reagent that fulfills the limitations of the claims. The examiner states that claim 7 is not allowable because the only support that is found for a reagent that produces a transfection efficiency greater than that obtainable by means of electroporation is Exgen 500 and therefore it would be remedial to limit the claims to this specific reagent. This rejection is respectfully traversed.

All of the independent claims have now been amended so as to require the use of a transfection reagent of the subclass of transfection reagents exemplified by Exgen 500. The examiner acknowledges that there is written description support for the use of Exgen 500. The specification states that Exgen 500 is in the subclass of transfection reagents known as "cationic non-lipid polymer transfection reagents". See page 12, lines 5-6 and lines 11-12 of the present specification. As Exgen 500 was selected as an example of this subclass of polymers, it is clear that applicant had the conception that any transfection reagent within this subclass of cationic non-lipid polymer transfection reagents would behave as Exgen 500. Furthermore, the claims are written in such a way that they would not encompass use of any cationic non-lipid polymer transfection reagent that did not provide a transfection efficiency greater than that obtainable by electroporation. Accordingly, all of the present claims are directed toward the exemplified embodiment that the examiner agrees is supported by the specification.

Claims 7, 66 and 71 are dependent claims that are more specifically directed to the Exgen 500 species, which is disclosed in this specification as being a linear polymer ethyleneimine, noting the present specification at page 12, lines 5-6. See also the Fermentas Life Sciences brochure where "Exgen 500 Universal Transfection Reagent" (2003), which is

submitted herewith. This brochure confirms that Exgen 500 is a 22 kDa linear polyethyleneimine. Thus, claims 7, 66 and 71 are directed specifically to Exgen, which the examiner conceded is free of this rejection. Furthermore, new independent claims 72-74 have now been added specifically directed to the use of Exgen, i.e., a linear polymer of polyethyleneimine. These claims omit any reference to electroporation. The specification shows the unexpected results obtained with this transfection reagent and therefore it is unnecessary to mention any kind of comparison with the prior art in these claims. Accordingly, at least claims 7, 66 and 71-74 should be indicated as being free of the present rejection. Reconsideration and withdrawal of this rejection are therefore respectfully urged.

Claims 1-9, 11-17 and 59-70 have been rejected under 35 USC 112, first paragraph, as failing to comply with the written description rejections. The Examiner states that the claims are directed to broadly using any transfection reagent which results in a transfection efficiency greater than that obtainable by electroporation, while the specification teaches that only Exgen provides a transfection efficiency greater than electroporation. The examiner states that the breadth of the genus of "cationic non-lipid polymer reagent" lacks a written description. The examiner states that the skilled artisan cannot envision the detailed chemical structure of all the

reagents that are encompassed by the claims, particularly as the as-filed disclosure provides only a single species, Exgen, in the genus of cationic non-lipid polymer reagents that results in the claimed effect. This rejection is respectfully traversed.

As discussed above, the claims have now been amended so as to be directed only to the subclass of transfection reagents that are cationic non-lipid polymer reagents, of which Exgen is an example. Submitted herewith is the following article: Bonetta, "The Inside Scoop-Evaluating Gene Delivery Methods", *Nature Methods*, 2:875-883 (2005). This article describes gene delivery methods, including electroporation, as well as the various classes of transfection reagents. Note that it includes liposome transfection agents, lipid-based transfection agents, and polymer transfection agents. As can be see on page 877, Exgen 500 reagent is discussed and referred to as being in the class of cationic polymers such as poly(lysine) and poly(ethyleneimine) (PEI). Thus, it is apparent that the three sub-classes of transfection reagents discussed in the present specification represent well-defined subclasses of transfection reagents that are art-recognized. Because the example of the cationic non-lipid polymer subclass worked very well, whereas examples of the other subclasses did not work as well and were no better than electroporation, it is reasonable to believe that applicant was in the profession of the entire

subclass of cationic non-lipid polymer transfection reagents, of which Exgen is an example.

While claims 7, 66 and 71-74 should definitely be free of the new written description rejection, in view of the fact that they are specifically directed to the Exgen material, the independent claims should also be allowable as there is no reason to believe that other transfection reagents in the subcategory of cationic non-lipid polymer transfection reagents should not all be operable in the same manner that Exgen 500 is operable. This is particularly true in view of the fact that the claims (other than new claims 72-74) only read on those cationic non-lipid polymer transfection reagents that have a transfection efficiency greater than that obtainable by electroporation. Accordingly, reconsideration and withdrawal of this rejection are respectfully urged.

Claims 1-9, 11-17 and 59-70 have been rejected under 35 USC 112, first paragraph, as failing to comply with the enablement requirement. The examiner states that the phrase "by means of electroporation" is not specifically defined in the specification and the working examples do not provide the specific parameters that are used to determine transfection efficiency using electroporation relative to the other claimed reagents. The examiner states that the efficiency of the electroporation depends on factors such as the number of pulses

and the intensity of the electric field. The examiner states the means of the electroporation of the claimed invention encompass conditions wherein the lower limit threshold would not allow any cells to uptake DNA and the upper limit threshold wherein the cells would be killed. This rejection is respectfully traversed.

The present specification discusses electroporation at page 11, lines 28-30. There it states that in murine ES cells, electroporation was found to be the method of choice for introducing foreign DNA into ES cells and Thomas et al (1987) *Cell*, Vol. 51, pp. 503-512 is cited with respect to this method of choice. Submitted herewith is a copy of the Thomas publication. The electroporation method using this publication is described in the paragraph bridging the two columns of page 511. There, it is clear that the electroporation was accomplished using the Promega Biotech X Cell 2000 and that the cells and the recombinant vector were exposed "to a single 265 V/cm pulse at room temperature." It is further noted that the present specification at page 17 line 32 states, "All references described in this description are herein incorporated by reference." Accordingly, as the Thomas reference has been incorporated by reference, the conditions recited therein have now been physically inserted into the present specification in order to quantify the preferred means of electroporation. Thus,

the specification at page 11, line 30, has been amended to add the following sentence:

> In the Thomas et al (1987) publication, electroporation is effected using the Promega X Cell 2000, exposing the cells and the vector to a single 625 V/cm pulse at room temperature.

This is not new matter as it is merely physically inserting into this specification that which that had been previously incorporated by reference.

The claims have also been amended everywhere that electroporation is mentioned to specify that the electroporation conditions are using a single 625 V/cm pulse at room temperature. It is believed that this amendment to the specification and the claims obviates the new enablement rejection as the claims now specify the number of pulses and the intensity of the electric field. While it is believed that the claims were fully enabled without this addition, nevertheless, by specifying a particular electroporation condition, it is now clear exactly how one could determine the metes and bounds of the claim, thus obviating the enablement rejection. Reconsideration and withdrawal of this rejection are therefore respectfully urged.

Claims 1-4, 6, 8, 9, 11-16, 36, 59-61, 65 and 67-70 have been rejected under 35 USC 103 as being unpatentable over Smith when taken with Ritter. This rejection is respectfully traversed.

It is noted that claims 7 and 66 have not been subject to this rejection. These claims specify that the transfection reagent is a cationic non-lipid polymer transfection reagent. All of the independent claims have now been amended to insert the subject matter previously appearing in claims 7 and 66. Accordingly, all of the present claims are now free of this rejection for the same reasons that claims 7 and 66 had been indicated to be free thereof. Reconsideration and withdrawal of this rejection are respectfully urged.

Claims 1-4, 6, 9, 11-13, 15, 16, 65, and 67-70 have been rejected under 35 USC 103 as being unpatentable over Smith when taken with the Gibco BRL. This rejection is respectfully traversed.

Again, it is noted that claims 7 and 66 have not been subject to this rejection. As all the claims now present in the case now have the feature previously recited in claims 7 and 66, none of the present claims are subject to the present rejections. Reconsideration and withdrawal thereof are therefore respectfully urged.

Claims 5 and 14 have been rejected under 35 USC 103(a) as being unpatentable over Smith when taken with Ritter and further in view of Myers. This rejection is respectfully traversed.

Claims 5 and 14 are patentable for the same reason as the claims from which they depend, discussed above. Accordingly reconsideration and withdrawal of this rejection are respectfully urged.

Claim 17 has been rejected under 35 USC 103(a) as being unpatenable over Smith when taken with Ritter and further in view Pascolo. This rejection is respectfully traversed.

Claim 17 is patentable for the same reason as the claim from which it depends, as discussed above. Accordingly, reconsideration and withdrawal of this rejection are also respectfully urged.

It is submitted that all of the claims now present in the case clearly define over the references of record and fully comply with 35 U.S.C 112. Reconsideration and allowance are therefore earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By /rlb/
Roger L. Browdy
Registration No. 25,618

RLB:tg
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
G:\BN\L\LUZZ\Benvenisty5\Pto\2007-07-11Amd.doc